

**Claims:**

1. A method for identifying, analyzing and/or cloning nucleic acid isoforms comprising the steps of:

- a) preparing at least two nucleic acid isoforms,  
complementary to each other;
- b) hybridizing the at least two complementary nucleic acid isoforms and forming double strand RNA/RNA or DNA/DNA hybrids comprising unpaired regions;
- c) recovering the RNA/RNA or DNA/DNA hybrids  
comprising unpaired regions from not hybridized nucleic acids and from nucleic acids not comprising unpaired regions ;
- d) identifying, analyzing and/or cloning the recovered nucleic acid fragment comprising unpaired regions.

2. The method of claim 1, wherein the recovering of step c) is carried out by using at least one restriction enzyme which cuts free single strand nucleic acids but does not cut double strand nucleic acids and/or at least a restriction enzyme which cut double strand nucleic acids but does not cut unpaired regions.

3. The method of claim 2, wherein the restriction enzyme which cuts free single strand nucleic acids but does not cut double strand nucleic acids is Exo VII, Exonuclease I, Exonuclease T, Lambda Exonuclease, T7 Exonuclease.

4. The method of claim 2, wherein at least one restriction enzyme which cuts double strand nucleic acids but does not cut unpaired regions is used.

5. The method of claim 4, wherein two restriction enzymes are used.

6. The method of claims 4-5, wherein the restriction enzymes cut at recognition sites comprising of 4 nucleotides of double strand nucleic acids but do not cut unpaired regions.

7. The method of claim 6, wherein the restriction enzymes are selected from HapII, HypCH4IV, AciI, HhaI, MspI, AluI, BstUI, DpnII, HaeIII, MboI, NlaIII, RsaI, Sau3AI, Taq alpha I and Tsp 509I.

5        8. The method of claims 1-7, wherein hybrids of RNA/RNA or DNA/DNA comprising unpaired regions are recovered from hybrid nucleic acids not comprising unpaired regions by using single strand nucleic acid-binding molecule.

10       9. The method of claim 8, wherein the single strand nucleic acid-binding molecule is bound to a tag.

10       10. The method of claims 8-9, wherein the nucleic acid to be recovered/single strand nucleic binding molecule/tag complex is recovered by use of a matrix which binds the tag.

15       11. The method of claim 8, wherein the single strand nucleic acid-binding molecule is a single strand nucleic acid-binding protein, antibody, antigen, oligonucleotide, a chemical group or chemical substance.

12. The method of claim 11, wherein the oligonucleotide which binds the tag is a random oligonucleotide.

20       13. The method of claim 12, wherein the random oligonucleotide is 15-30 nucleotides.

14. The method of claim 13, wherein the random oligonucleotide is 25 nucleotides.

25       15. The method of claim 8-14, wherein the tag is biotin, digoxigenin, antibody, antigen, a protein or nucleic acid binding molecule and the matrix is avidin, streptavidin, digoxigenin-binding molecule, an antibody or its ligand and/or chemical matrix.

30       16. The method of claims 8-15, wherein the tag is digoxigenin the matrix is a digoxigenin-binding molecule

17. The method of claims 8-15, wherein the tag is biotin and the matrix is avidin or streptavidin.

18. The method of claims 8-17, wherein the single strand nucleic acid-binding molecule is covalently attached to the matrix.

19. The method of claims 8-18, wherein the matrix is associated to a solid matrix surface selected from the group consisting of metal beads, magnetic beads, inorganic polymer beads, organic polymer beads, glass beads and agarose beads.

20. The method of claims 1-19, wherein hybrids of RNA/RNA or DNA/DNA comprising unpaired regions are recovered from hybrid nucleic acids and released from the single strand nucleic acid-binding molecule.

21. The method of claims 1-20, wherein the recovered nucleic acids comprising unpaired regions are bound with Y-shaped oriented linkers comprising a sticky end.

22. The method of claim 21, wherein the Y-shaped oriented linker comprises a different marker sequence in each single strand arm.

23. The method of claims 21-22, wherein the Y-shaped linker comprises a sticky end which hybridized with the sticky end of the fragment comprising the unpaired region.

24. The method of claim 23, wherein the sticky end of the Y-shaped linker hybridizes to the sticky end of the fragment comprising the unpaired region cut by the restriction enzymes of claims 4-7.

25. The method of claims 1-24, wherein the at least two nucleic acid isoforms are prepared from at least one nucleic acid library, biological sample, cell, tissue, organ or biopsy.

26. The method of claim 25, wherein the two nucleic acid isoforms are prepared from two or more different nucleic acid libraries, biological samples, cells, tissues, organs or biopsies.

27. The method of claims 25-26 wherein the at least one

of the at least two nucleic acid libraries, biological samples, cells, tissues, organs or biopsies is from tumoral source, from treated cells, and/or from cells undergoing apoptosis.

28. The method of claims 1-27, wherein the nucleic acids  
5 comprising nucleic acids comprising unpaired regions as recovered at step a), b), c) and/or d) of claim 1, are stored as nucleic acid isoforms-enriched libraries, used for the analysis of isoforms, or clones and/or used for the detection of further isoforms.

10 29. The method of claim 28, wherein the obtained libraries are alternative splicing-enriched libraries.

30. The method of claims 1-29, wherein the recovered nucleic acids comprising unpaired regions are amplified and cloned.

15 31. The method of claims 1-30, wherein the unpaired regions correspond to portions of genes that are differentially spliced.

20 32. The method of claims 1-31, wherein the unpaired regions correspond to portions of related genes derived from different loci within the same genome.

33. The method of claims 1-31, wherein the unpaired regions correspond to portions of unrelated genes derived from the same locus within a genome.

25 34. The method of claims 1-31, wherein the unpaired regions correspond to portions of related genes derived from different genomes.

35. The method of claims 1-34, wherein the recovered and cloned nucleic acid comprise the whole sequence of an unpaired region.

30 36. The method of claim 35, wherein the unpaired region corresponds to an exon or intron.

37. The method of claims 1-36, wherein the at least two

complementary nucleic acid isoforms are prepared from starting materials by using at least two different RNA and/or DNA polymerases wherein each of the polymerases recognizes a different promoter site.

5           38. The method of claim 37, wherein RNA transcripts are obtained from the starting materials by using RNA polymerases which recognize a different promoter site, and cDNAs are prepared from the RNA transcripts by using reverse transcriptase.

10           39. The method of claim 38, wherein the at least two RNA polymerases recognizing different promoter site are selected from the group consisting of T3 RNA polymerase, T7 RNA polymerase, SP6 RNA polymerase and K11 RNA polymerase.

15           40. The method of claims 1-39, wherein a DNA polymerase and strand specific primers are used.

          41. The method of claims 1-39, wherein a DNA polymerase and strand specific primers are used for linear amplification.

20           42. The method of claims 40-41, wherein the DNA polymerase is Taq DNA Polymerase or DNA Polymerase I Large (Klenow) Fragment, Exonuclease Minus

          43. The method of claim 1, wherein the in step c) the nucleic acid isoforms are recovered by using linkers or primers.

25           44. The method of claim 43, wherein the linker or primer recognizes specific sequence sites.

          45. The method of claim 43, wherein the isoform nucleic acids are recovered by using a linker and DNA or RNA ligase.

          46. The method of claim 45, wherein the ligase is T4 DNA ligase, E.coli DNA ligase, RNA ligase or T4 RNA ligase.

30           47. The method of claims 1-46, wherein vectors or primers are used to introduce recognition sites for the 4bp cutters restriction enzymes of claims 4-7 at the ends of the nucleic

acid isoforms.

48. The method of claims 1-47, wherein the nucleic acid isoforms are prepared from fragmented genomic DNA, cDNA, full-length cDNA, mRNA and/or RNA.

5 49. The method of claims 1-48, wherein the isoforms are full-length cDNAs or a fragment thereof comprising the unpaired region.

50. The method of claim 49, wherein the isoform substantially comprises the unpaired region.

10 51. A cloning vector comprising the isoform obtained according to the method of any one of claims 1-50.

52. A host cell comprising the vector of claim 51.

53. A method for the preparation of isoform polypeptides comprising preparing the culture host cell of claim 52

15 54. A method for preparing an isoform polypeptide comprising the step of preparing a isoform nucleic acid according to claims 1-50 and preparing the corresponding isoform polypeptide by using free-cell in-vitro or in vivo system.

20 55. A method for the identification of isoform polypeptides using the information obtained according to the method of claims 1-54.

56. A method for the detection and/or isolation of nucleic acid isoforms comprising the steps of:

25 1) preparing at least one oligonucleotide probe comprising the whole or part of sequence of an unpaired region identified and/or cloned according to claims 1-50; and

m) hybridizing the oligonucleotide probe to nucleic acids comprising nucleic acid isoforms;

30 n) isolating the nucleic acid isoforms.

57. The method of claim 56, wherein the oligonucleotide probe is used to isolate full-length nucleic acid isoform.

58. The method of claims 56-57, wherein the oligonucleotide probe comprise at least part of or the entire sequence of one exon or intron.

59. A method for the determination of sequence variation  
5 of isoforms of claims 1-58, comprising the full-length or partial sequencing of the isoform.

60. The method of claims 1-59, wherein the sequence information of the sequence isoforms is used for the design of sequencing primers.

10 61. The method of claims 1-59, wherein the obtained isoform sequencing data are aligned to the genome, to genomic sequencing data and/or to cDNA sequencing data to obtain genetic information.

62. The method of claim 61, wherein the information in on  
15 alternative splicing.

63. The use of information obtained from claims 1-62, for the preparation of a nucleic acid probe.

64. The nucleic acid probe of claim 50.

65. Use of the information obtained from the method of  
20 claims 1-63, for the detection and/or diagnosis of a disease, disease condition, pathology, a physiological condition, for assessing toxicity, for assessing the therapeutic potential of a test compound and/or for assessing the responsiveness of a patient to a test or treatment.

25 66. Method for recovering of full-length cDNAs from cDNA libraries, biological samples, cells, tissues, organs or biopsies, from tumoral source, from treated cells, and/or from cells undergoing apoptosis by using the information on alternative splicing of claim 62.

30 67. Method for recovering of full-length cDNAs according to claims 1-66 from cDNA libraries, biological samples, cells, tissues, organs or biopsies is from tumoral source, from

treated cells, and/or from cells undergoing apoptosis by using the information on alternative splicing.

68. Use of isoforms obtained according to the method of claims 1-52 and/or the nucleic acid probe of claim 64 for the  
5 preparation of non-soluble supports for hybridization in situ.

69. A non soluble support comprising at least an nucleic acid comprising an unpaired region prepared according to the method of claims 1-62, a nucleic acid complementary to the unpaired region and/or the probe of claim 64, fixed, applied  
10 and/or printed thereon.

70. The support of claims 68-69, which is a solid matrix.

71. The support of claims 68-69, which is a microarray.

72. Use of the support of claims 68-71, for the identification and isolation of nucleic acid isoform.

15 73. Use of the support of claims 68-71, for in situ hybridization.

74. Use of the support of claims 68-71, for the detection and/or diagnosis of a disease, disease condition, pathology, a physiological condition, for assessing toxicity, for assessing  
20 the therapeutic potential of a test compound, for assessing the responsiveness of a patient to a test or treatment, for the detection of nucleic acids and/or for the detection of nucleic acid isoforms.

75. Use of genetic information obtained according to  
25 claims 1-74 for detecting and/or isolating nucleic acids from a support, microarray, nucleic acid library, biological sample, cell, tissue, organ and/or biopsy.

76. A computer program and/or software applied on a medium for the analysis of genetic information obtained  
30 according to claims 1-75.

77. A computer program and/or software applied on a medium for the alignment of the nucleic acid isoforms



sequences or information obtained according to claims 1-76 to genomic and/or cDNA sequence information.

78. A computer program and/or software applied on a medium for the prediction, determination and/or analysis of  
5 functional domains of polypeptides that derive from nucleic acid isoforms sequence or information obtained according to claims 1-77.